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The Heterogeneity of Systemic Inflammation in Bronchiectasis

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Abstract

BACKGROUND: Systemic inflammation in bronchiectasis is poorly studied in relation to aetiology and severity. We hypothesized that molecular patterns of inflammation may define particular aetiology and severity groups in bronchiectasis.

METHOD: We assayed blood concentrations of 31 proteins from 90 bronchiectasis patients (derivation cohort) and conducted PCA to examine relationships between these markers, disease aetiology and severity. Key results were validated in two separate cohorts of 97 and 79 patients from other centres.

RESULTS: There was significant heterogeneity in protein concentrations across the derivation population. Increasing severity of bronchiectasis (BSI) was associated with increasing fibrinogen ($\rho = 0.34$, $p = 0.001$ –validated in a second cohort), and higher fibrinogen was associated with worse lung function, *Pseudomonas* colonisation and impaired health-status. There were generally similar patterns of inflammation in patients with idiopathic and post-infectious disease. However, patients with primary immunodeficiency had exaggerated IL-17 responses, validated in a second cohort ($n = 79$, immunodeficient 12.82pg/ml versus idiopathic/post-infectious 4.95pg/ml, $p = 0.001$), and thus IL-17 discriminated primary immunodeficiency from other aetiologies (AUC 0.769 (95%CI 0.661-0.877)).

CONCLUSION: Bronchiectasis is associated with heterogeneity of systemic inflammatory proteins not adequately explained by differences in disease aetiology or severity. More severe disease is associated with enhanced acute-phase responses. Plasma fibrinogen was associated with bronchiectasis severity in two cohorts, *Pseudomonas* colonisation and health status, and offers potential as a useful biomarker.

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Introduction

Bronchiectasis is a neglected chronic inflammatory airway condition that is the end anatomical result of diverse aetiological insults. Whilst it has been known for some time that bronchiectasis is also associated with systemic inflammation [1], the nature of this response has not been well studied in relation to bronchiectasis aetiology and severity. Importantly, no previous studies have examined whether inflammation differs between major causes of bronchiectasis such as idiopathic and post-infectious disease. Indeed, there are no existing data examining a comprehensive panel of inflammatory proteins in the systemic compartment of patients with bronchiectasis.

Current concepts of bronchiectasis severity emphasise the importance of multi-component assessment [2, 3]. Scores such as the Bronchiectasis Severity Index (BSI) [2] and Faced [3] predict mortality, hospital admissions, exacerbation frequency and quality of life. There is also a paucity of data examining causes of death in bronchiectasis despite a suggestion of increased cardiovascular risk [4]. This is a concept well established in other chronic airway diseases such as chronic obstructive pulmonary disease (COPD) and which may relate to systemic inflammation [5]. There is therefore the need to examine how bronchiectasis severity is associated with systemic inflammation and, in particular, which components of bronchiectasis severity drive this association.

We designed a study to address the hypotheses that systemic inflammatory markers can distinguish aetiology and severity groups in bronchiectasis, employing an unsupervised principal component analysis. In doing so we have conducted the largest and most comprehensive analysis of systemic inflammatory proteins in bronchiectasis ever reported.

Moreover, we validated key results in two replication cohorts. Our results inform on the heterogeneity of systemic inflammatory responses in bronchiectasis with regard to aetiology and severity of disease.

Method

90 patients with stable, clinically significant bronchiectasis – regular sputum production and/or recurrent respiratory infections with a diagnostic computed tomography (CT) scan - were recruited as a convenience sample from out-patient clinics at the Royal Free London and University College London Hospitals NHS Foundation Trusts, UK. Patients with a primary clinical diagnosis of COPD were excluded. These patients formed the derivation cohort. Patients were only recruited if free from exacerbation for one month or more. Patients provided written informed consent, and the study was approved by Hampstead Research Ethics Committee (10/H0720/43).

1. Clinical Assessment

For the purposes of this analysis, information was collated to permit allocation of patients into aetiological groups according to the British Thoracic Society guideline [6]. Idiopathic disease was defined as the absence of an alternative more plausible cause of bronchiectasis.

Bronchiectasis severity was first assessed using the Bronchiectasis Severity Index [2] and therefore data on age, body mass index (BMI), most recent lung function (forced expiratory volume in 1 second, FEV₁), self-reported hospitalisation (previous two years) and exacerbations (previous year), Medical Research Council (MRC) dyspnoea scale, the

presence of colonising organisms on routine sputum culture and radiological extent of disease on the most recent CT scan were collated and entered into the online BSI calculation tool: <http://www.bronchiectasisseverity.com/>. Mild bronchiectasis was defined as a score of 0-4, moderate as 5-8 and severe as ≥ 9 units. To provide further information in relation to bronchiectasis severity, we also calculated the alternative FACED bronchiectasis severity score [3], which includes age, FEV₁, lobar involvement, dyspnoea (mMRC score) and *Pseudomonas* colonisation status. The FACED score is as follows: mild 0–2 points, moderate 3–4 points and severe 5–7 points.

2. Assessment of Serum Proteins

Venous blood samples were drawn. A citrated plasma sample was analysed for fibrinogen using the Clauss method (IL ACL Top Coagulation Analyzer; Instrumentation Laboratories, Lexington, MA). A serum sample was analysed for C-Reactive Protein using a Modular Analytics E 170 Module (Roche, Burgess Hill, UK). A second serum sample was centrifuged (10 minutes, 2000rpm, 4°C) with the supernatant then stored at -80°C for later analysis of the remaining 29 mediators (see Table 1). These were assessed using the Meso Scale Discovery Platform 'V-PLEX Human Cytokine 30-Plex Kit' (Meso Scale Discovery, Rockville, MD, US) according to the manufacturer's instructions.

3. Validation Cohorts

Key results were validated in two samples of patients from specialist bronchiectasis clinics in Dundee and Newcastle, UK. These patients had clinically significant bronchiectasis and also underwent standardised testing according to BTS recommendations, as in the derivation cohort. Ethical approval was granted (12/ES/0059 and 12/NE/0248 respectively).

Fibrinogen was measured by the Clauss method (Dundee patients). Serum IL-17 (Newcastle patients) was measured using a commercial quantitative enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's protocol (DuoSet® ELISA Development System, human IL-17, R&D Systems, UK). Health status as assessed by the St. George's Respiratory Questionnaire (SGRQ) was available in the Dundee patients.

4. Statistical Analysis

Statistical analysis was performed in IBM SPSS version 22 (IBM Corporation, Armonk, NY, US). Data were tested for normality using the Kolmogorov-Smirnov test. Clinical data are reported as mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate.

The majority of the serum proteins were not normally distributed and therefore all were \log_{10} transformed for further analysis. As described in Table 1, any assays below the detection limit were assigned a value of half that lower limit of detection (LLD). 17 missing fibrinogen results in the derivation cohort arising because no plasma sample was available were single-imputed based on a significant correlation between fibrinogen and CRP ($\rho=0.572$, $p<0.001$). We validated a key finding in relation to fibrinogen using minimum, mean and maximum values derived from multiple imputation using the 'mi' command in STATA version 14 (www.stata.com).

We used Principal Component Analysis (PCA) [7] to assess variation in the proteins across the population. The protein most closely correlated with each of the first five principal components was selected as a key exemplar protein for that principal component.

Radar plots [8] were employed to illustrate the concentrations of these five key proteins across disease severity and aetiology. To do this, the concentration of each of these proteins was expressed as the quartile of the concentration it was contained in, derived from the concentrations across all 90 patients. The mean of each of these quartile values was then used to construct the radar plot.

Correlations were assessed using Pearson or Spearman Rank as appropriate, and differences between medians tested by Mann-Whitney U analysis for two groups, and Kruskal–Wallis one-way analysis of variance when more than two groups were tested. We also employed multiple linear regression and receiver-operating characteristic (ROC) analysis.

A p value <0.05 was considered statistically significant.

Results

The 90 patients (57, 63% women) in the derivation cohort had a median (IQR) age of 61 (46-71) years, BSI score of 5 (3-8) units and FACED score of 2 (1-3) units. Within the variables contributing to the BSI, the mean (SD) FEV₁ was 72.4 (27.5) % predicted, and exacerbation frequency 2 (1-3) events/year. The three largest aetiological groups were post-infection (18 patients), idiopathic (20 patients) and primary immunodeficiency (25 patients, all of whom had CVID) which together accounted for 70% of the cohort in total. A sputum culture result within six months of the sampling visit was available in 30/90 patients. Patients were additionally asked if they recalled a positive sputum culture over the previous year where samples had been sent to referring hospitals. Regarding therapy, 37 patients were prescribed an inhaled corticosteroid, 18 a long-term macrolide, 19 a long-term antibiotic other than a macrolide, eight were prescribed long-term oral corticosteroids and seven long-term immunosuppression other than corticosteroids. The results of analysis of the 31 systemic proteins are reported in Table 1.

TABLE 1: Results of the analysis of 31 proteins in 90 patients with bronchiectasis from the derivation cohort. All results are expressed as geometric mean (SD) pg/ml except Fibrinogen (g/l) and CRP (mg/l).

(all logs)	n= above the lower limit of detection	Mean (SD)
CRP	90	2.8 (3.9)
Eotaxin	90	147.9 (1.9)
Eotaxin-3	73	14.1 (3.3)
Fibrinogen	90	3.5 (1.4)
GM-CSF	83	0.4 (2.3)
IFN- γ	84	9.1 (3.4)
IL-1 α	41	0.4 (6.2)
IL-1 β	53	0.1 (4.3)
IL-2	45	0.3 (4.3)
IL-4	37	0.1 (3.4)
IL-5	86	0.5 (2.6)
IL-6	86	1.3 (2.5)
IL-7	90	15.1 (1.6)
IL-8	90	13.5 (2.8)
IL-10	78	0.4 (3.2)
IL-12p40	90	107.2 (2.2)
IL-12p70	65	0.7 (2.3)
IL-13	63	6.9 (1.7)
IL-15	90	2.3 (1.4)
IL-16	90	257.0 (1.6)
IL-17	90	4.0 (2.6)
IP-10	90	309.0 (2.5)
MCP-1	90	263.0 (1.8)
MCP-4	90	81.3 (1.6)
MDC	90	831.8 (1.7)
MIP-1 α	83	21.9 (1.6)
MIP-1 β	90	112.2 (1.6)
TARC	90	275.4 (2.1)
TNF- α	90	1.8 (1.6)
TNF- β	79	0.2 (2.8)
VEGF	90	109.6 (1.9)

Values below the lower limit of detection were assigned a value of half that lower limit.

1. Principal Component Analysis

Great heterogeneity was observed in the analysis of systemic protein markers across the dataset. An unsupervised principal component analysis met the criteria for data adequacy (Bartlett's test of Sphericity and Kaiser-Meyer-Olkin measure of sample adequacy) and yielded 10 factors with an Eigen value > 1. Monte-Carlo parallel analysis based on a

theoretical population of 100 subjects suggested a five factor solution, which then explained only 52% of the variance. Table 2 presents the five extracted factors, and the two serum proteins most closely associated with those factors. Principal component 1, the component associated with the greatest variance across the derivation cohort, was most closely correlated with Monocyte Chemoattractant Protein (MCP)-1.

TABLE 2: *Principal Component analysis of 31 proteins in the blood of 90 patients with bronchiectasis in the derivation cohort identified five components that explained 52% of variance. The number in brackets represents the strength of the association between that marker and the respective principal component.*

Component	Nature	Correlates
1	'Mononuclear Chemotaxis'	MCP-1 (0.841); MDC (0.773)
2	'Immune Regulation'	IL-13 (-0.759); IL-12p70 (-0.729)
3	'Acute Phase Response'	Fibrinogen (0.719); CRP (0.711)
4	'Immune Activation'	IL-17 (0.796); IL-12p40 (0.596)
5	'Polymorphonuclear Chemotaxis'	IL-8 (0.638); IL-16 (0.633)

We next went on to explore differences by disease severity (BSI and FACED score) and aetiology in patterns of these components, using the first key cytokine associated with that component.

2. Bronchiectasis Severity and Systemic Inflammatory Proteins

Figure 1A presents a Radar Plot of the five key systemic inflammatory proteins (representing each of the Principal Components) by bronchiectasis severity (BSI score) in the derivation cohort. To mitigate heterogeneity due to aetiology, Figure 1B presents data solely from the 18 patients with post-infectious disease (likely to be the most homogeneous group).

In both the total derivation cohort and the selected patients with post-infectious aetiology, there was a step-wise increase in fibrinogen from mild through moderate to severe patients,

but significant heterogeneity in the other four markers. Reflecting this, in the whole derivation cohort, we observed a significant correlation between the plasma fibrinogen concentration and BSI score ($\rho=0.34$, $p=0.001$, Figure 2A). This was robust to analysis using minimum, mean and maximum fibrinogen values derived from multiple rather than single (CRP-based) imputation ($r=0.23$ $p=0.03$; $r=0.37$ $p<0.001$ and $r=0.41$ $p<0.001$ respectively). A similar relationship was present between bronchiectasis severity assessed by the FACED score and plasma fibrinogen concentration ($\rho=0.27$, $p=0.014$, Figure 2B). There were no significant correlations between BSI score and the other four markers.

We sought to validate the relationship between BSI and plasma fibrinogen by replicating this finding in a separate cohort. Fibrinogen concentration was available in 97 patients from Dundee, UK. These patients had a median (IQR) age 65 (58-72) years and FEV₁ 1.72 (1.11-2.48)l, 50% were female and the two commonest aetiologies were idiopathic (46%) and post-infectious (19%) disease. Only three patients in this cohort had primary immunodeficiency. The median (IQR) BSI was 7 (4-12) and fibrinogen 3.6 (2.7-4.8) g/l. There was a significant relationship between BSI and fibrinogen in this second independent group of patients ($\rho=0.36$, $p<0.001$), of strikingly similar strength to that in the derivation cohort thus demonstrating the finding to be robust. There was also a significant relationship between plasma fibrinogen and the St. George's Respiratory Questionnaire ($\rho=0.25$, $p=0.013$), indicating that fibrinogen also reflects health-status in bronchiectasis.

Finally, we examined which components of the BSI score were most closely associated with the plasma fibrinogen concentration by conducting a multi-variable analysis with fibrinogen as the dependent variable and each of the BSI components included as predictors (using the

allocated BSI 'points' rather than raw values [2]). This was conducted in the combined UCL and Dundee cohorts of 187 patients. The overall model fit (R^2)=0.17, and the variables significantly associated with (higher) plasma fibrinogen were (greater) age (β =0.201; p =0.006), (lower) FEV₁ (β =0.178; p =0.030) and (the presence of) *Pseudomonas* colonisation (β =0.170; p =0.039). 20 of the 187 patients (10.7%) in the combined cohort were *Pseudomonas* colonised.

3. Bronchiectasis Aetiology and Systemic Inflammatory Proteins

Figure 3A represents a Radar Plot of the five key systemic inflammatory proteins (representing each of the Principal Components) by the three commonest bronchiectasis aetiologies in the whole derivation cohort. To mitigate heterogeneity due to severity, Figure 3B presents data solely from the 25 patients with moderate disease (BSI score 5-8).

Whilst the profiles of the post-infectious and idiopathic patients look similar, patients with primary immunodeficiency appear to have up-regulated IL-17 responses. Reflecting this, the median log₁₀ serum IL-17 concentration was significantly different across the three groups (idiopathic / post-infectious / primary immunodeficiency 0.45 vs. 0.45 vs. 0.82 log₁₀ pg/ml, p <0.001), but there were no significant differences for the other four markers by bronchiectasis aetiology (MCP p =0.439, IL-13 p =0.223, Fibrinogen p =0.138 and IL-8 p =0.549).

Next, we went on to test the principle that IL-17, as an exemplar serum protein, might be of practical use in the diagnosis of bronchiectasis aetiology. Figure 4 presents a Receiver

Operating Characteristic (ROC) curve for the ability of IL-17 to distinguish primary immunodeficiency from idiopathic and post-infectious aetiology. The area under the curve ((AUC)=0.769 (95%CI 0.661-0.877, $p<0.001$)) approaches the standard 0.8 cut off for utility, which falls within the 95%CI. Reporting geometric means, a serum IL-17 concentration of 3.23pg/ml would be 90% sensitive and 63% specific, and a concentration of 6.46pg/ml would be 90% specific and 50% sensitive for the identification of bronchiectasis associated with primary immunodeficiency. We have not reported positive and negative predictive values as these are dependent on prevalence.

We next sought to validate the difference in serum IL-17 between primary immunodeficiency versus idiopathic and post-infectious disease by replicating this finding in a separate cohort. Serum IL-17 concentration data were available in 79 patients from Newcastle, UK. These patients had a median (IQR) age 64 (61-70) years, FEV₁ 1.59 (1.07-2.06) l, and 65% were female. 50 patients had post-infectious disease, 19 had idiopathic disease and 10 patients had bronchiectasis associated with primary immunodeficiency. The median (IQR) serum IL-17 was 5.37 (2.43-12.19)pg/ml. There was a significant difference in serum IL-17 concentration between patients with primary immunodeficiency versus those with idiopathic/post-infectious disease (12.82 vs. 4.95pg/ml, $p=0.001$ respectively), thus demonstrating the derivation cohort finding to be robust.

Finally, as we were specifically interested in differences in protein expression in patients with idiopathic versus post-infectious disease, we compared the median values of the remaining 26 markers across these two groups in the derivation cohort. The only significant difference was for IL-4, which was higher in the post-infectious than the idiopathic group ($p=0.025$),

but IL-4 was detectable in only 37 of 90 patients (Table 1). A chi square analysis examining detectable versus non-detectable IL-4 concentration by post-infectious versus idiopathic disease did not reach statistical significance (detectable 11/18 vs. 6/20 $p=0.101$). The AUC (95%CI) for the ROC of IL-4 to differentiate these two aetiologies was 0.694 (0.521-0.868), $p=0.041$.

Discussion

We present the most comprehensive analysis of systemic immune and inflammatory proteins from patients with bronchiectasis ever reported. Our findings may be summarised thus: first, there is significant heterogeneity in systemic proteins between patients which is not fully accounted for by differences in either bronchiectasis aetiology, or bronchiectasis severity. Second, a global assessment of bronchiectasis severity (and health status) is associated with an increasing systemic acute-phase response. Third, we demonstrate the important principle that profiles of serum inflammatory proteins may differ significantly between aetiology subgroups, but we were unable to demonstrate significant differences in patterns of proteins between idiopathic and post-infectious disease.

The classic vicious cycle hypothesis of Cole [9] presents (airway) inflammation as a key component of bronchiectasis. It has been known for some time that patients with bronchiectasis also present a systemic inflammatory response [1] including up-regulated expression of circulating leukocyte adhesion molecules [10] even when clinically stable. The former analysis of differential leukocyte count, CRP, erythrocyte sedimentation rate and serum immunoglobulins in 87 patients reported significant associations between systemic

inflammation and bronchiectasis severity as assessed both by CT scan and lung function. It was subsequently shown that systemic inflammation is elevated in patients with high airway bacterial load, and that this effect can be modified with antibiotic therapy [11]. We now recognise bronchiectasis as a complex disease which is best assessed using multi-component scores [2, 3]. However, there are no existing studies examining a comprehensive panel of systemic protein markers in patients with bronchiectasis, allowing an assessment of protein heterogeneity by severity and aetiology.

We first took an unsupervised principal component analysis of 31 candidate proteins representing a wide range of immune and inflammatory mechanisms and identified five components accounting for 52% of the variance. In broad terms, these components represented proteins involved in chemotaxis of mononuclear and polymorphonuclear leukocytes, proteins associated with immune regulation, immune activation, and components of the acute-phase response. To avoid problems resulting from a multiplicity of statistical tests, we went on to conduct further analysis only in the single protein most closely associated with each of the five principal components.

The finding that a five component PCA accounted for only around half of the total variance demonstrates the degree of heterogeneity in systemic protein responses. Most of the variance was seen in markers of mononuclear chemotaxis, exemplified by MCP-1, yet this variance was not explained by differences in either the aetiology or severity of disease. MCP-1 (CCL2) is a CC chemokine responsible for recruiting monocytes, T-lymphocytes and dendritic cells to sites of inflammation [12]. A role for MCP-1 has not previously been reported in bronchiectasis. Further work is required to explore the factors determining the

variance in this marker between patients, and its potential as an outcome marker or therapeutic target. Whilst bronchiectasis is usually considered a condition of principally neutrophilic inflammation, variance in serum IL-8 concentration was also unexplained by differences in bronchiectasis aetiology and severity. This is perhaps not surprising as recruitment and activation of neutrophils occurs locally in the airway and not in the systemic circulation.

With the possible exception of IL-4, we were unable to define differences in systemic protein responses between individuals with idiopathic and post-infectious bronchiectasis, the two commonest causes in clinical practice in the developed world. Further study of IL-4 in this context is warranted. However, differentiating between idiopathic and post-infectious bronchiectasis is challenging because patients classified as idiopathic may have had unreported respiratory infection, and where bronchiectasis is classified as post-infectious there may be recall bias with tenuous linkage to a previous, often childhood, infection. Anwar *et al* [13] suggested that post-infectious bronchiectasis may be a valid subgroup as patients had an earlier onset of disease, but evidence to date as to whether idiopathic and post-infectious disease are separate phenotypes remains inconclusive. Our data demonstrate that these aetiologies are difficult to differentiate in terms of systemic inflammatory profiles.

Our finding of greater IL-17 responses in patients with primary immunodeficiency was validated in an independent cohort. This raises the possibility that assessment of serum proteins may have utility in the differential diagnosis of bronchiectasis although prospective analysis would be required to confirm such a hypothesis. IL-17 has a key role in inducing

and mediating pro-inflammatory responses [14]. IL-17 is produced by a variety of immune cells, classically though not solely T-helper 17 cells, and acts on epithelial cell, endothelium and fibroblasts to result in release of pro-inflammatory mediators including IL-8, and drives neutrophil recruitment. Elevated airway IL-17 has been previously reported in patients with bronchiectasis. [15] In asthma, systemic IL-17 is elevated and airway IL-17 has been associated with more severe and neutrophilic asthma phenotypes (**Bullens DM, Truyen E, Coteur L, Dilissen E, Hellings PW, Dupont LJ, Ceuppens JL. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res* 2006; 7:135. And Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, Lemi`ere C, Hamid Q. T(H)17-associated cytokines (IL-17A and IL17F) in severe asthma. *J Allergy Clin Immunol* 2009;123: 1185–1187) while elevated airway IL-17 in COPD has been associated with lower FEV₁/FEV ratio.(*Chest*. 2010 Nov;138(5):1140-7. doi: 10.1378/chest.09-3058. Epub 2010 Jun 10. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, McCormick M, Woods J, May R, Sleeman MA, Anderson IK, Brightling CE.) That bronchiectasis in immunodeficiency may be associated with exaggerated as well as deficient responses is consistent with our earlier work defining greater airway inflammation and systemic CRP in bronchiectasis associated with primary antibody deficiency syndromes compared to immuno-competent bronchiectasis subjects [16]. Further work comparing systemic IL-17 between primary antibody deficient patients with and without bronchiectasis would help to evaluate a possible role of this pro-inflammatory cytokine in the development of bronchiectasis. Identification of IL-17 pathways is important as monoclonal drugs are available to target IL-17 and, indeed, macrolide antibiotics (which are effective at reducing**

exacerbations in bronchiectasis [17-19]) have been shown to reduce IL-17 expression in patients with bronchiectasis [20].

Global assessments of bronchiectasis severity (BSI and FACED score) were associated with an increasing acute-phase response, exemplified by plasma fibrinogen concentration, and we replicated this result in an independent cohort. This effect was driven largely by the reduction in lung function, *Pseudomonas* colonisation and age. Our findings are in keeping with Wilson's 1998 report of an association between lung function and systemic inflammation in bronchiectasis [1]. Systemic inflammation may provide the link between airway inflammatory diseases and the increased cardiovascular risk observed in such conditions [5], of clinical importance because many patients with COPD, for example, die from co-morbid cardiovascular disease [21]. As a coagulation protein, fibrinogen may be directly involved in cardiovascular risk. The concept of cardiovascular risk in bronchiectasis is poorly studied, but we have recently reported that patients with bronchiectasis are at increased risk of cardiovascular events compared to a control population [4]. Our results raise the hypothesis that preservation of lung function and, perhaps, prevention of *Pseudomonas* colonisation could be important strategies to mitigate the excess cardiovascular risk associated with bronchiectasis. The utility of biomarkers such as fibrinogen in adding to mortality prediction in COPD [22] has resulted in FDA consideration of this protein as a surrogate outcome measure in clinical trials in COPD [23]. There is therefore the need to understand further the role of fibrinogen in bronchiectasis.

A major strength of our study is that we have validated our two key findings in independent cohorts. However a limitation, common to all bronchiectasis studies, is that populations

vary across centres depending on local referral patterns. In our derivation population, for example, we were not able to explore systemic protein responses in bronchiectasis associated with other immune-dysregulated conditions such as rheumatoid arthritis. Whilst patients with idiopathic and post-infectious disease represent a sizeable proportion of the derivation cohort as one would expect in the UK, our clinical practice includes a tertiary immunology service and therefore patients with primary immunodeficiency are over-represented in the derivation cohort. For this reason, we have reported the sensitivity and specificity of IL-17 cut-points for diagnosing immunodeficiency-associated bronchiectasis, rather than positive and negative predictive values which depend on prevalence. Cost constraints would prohibit comprehensive unsupervised analysis in very large populations and therefore our principal component analysis provides useful data on which systemic proteins in bronchiectasis are responsible for most of the inter-patient variation, with the novel identification of MCP-1. A further potential use for systemic proteins is to assess disease progression, but this would require longitudinal rather than cross-sectional analyses. We were not able to study relationships between airway bacterial density or the microbiome and systemic proteins, a limitation of our work and this would be an important area for future research. All samples were taken from patients at least one month following a preceding exacerbation; it is possible that in some patients inflammatory responses may not have fully resolved. Furthermore, the range of anti-inflammatory and anti-infective medications used in our cohort prevent us from commenting on the effect of individual or combination therapies on systemic protein concentrations, which would therefore also be a further important area of future study.

In summary, we report significant heterogeneity in systemic inflammation between patients with bronchiectasis. This heterogeneity is not solely associated with either disease aetiology or severity. Indeed, the profiles of inflammation were generally similar in heterogeneity across the two most important aetiologies: idiopathic and post-infectious disease. A global index of bronchiectasis severity was associated with an increased acute-phase response, in two cohorts. This finding offers insight into a possible role for fibrinogen and increased cardiovascular risk in bronchiectasis. We also provide proof-of-principle that assessment of systemic proteins may support diagnosis of rarer, specific causes of bronchiectasis.

Declaration of Interests

None in relation to the published work.

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Figure Legends

FIGURE 1: Radar plots of five serum inflammatory proteins by severity of bronchiectasis in the whole derivation cohort (Figure 1A) and only those patients with post-infectious disease (Figure 1B). The green line represents mild disease (BSI score 0-4), orange line moderate disease (BSI score 5-8) and black line severe disease (BSI >9 units). Numbers refer to quartiles of (\log_{10}) serum concentration.

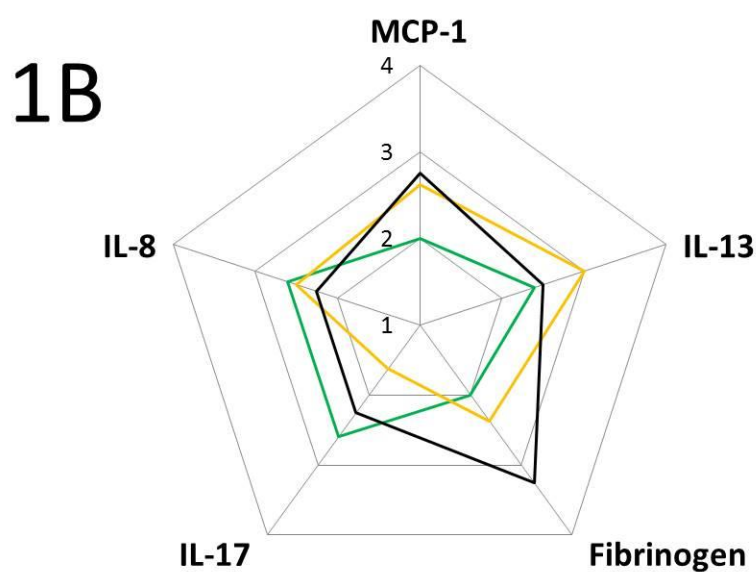
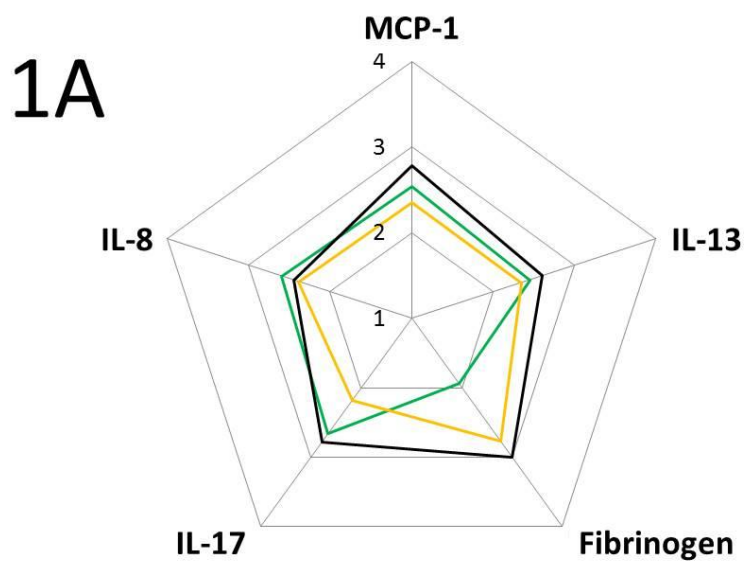
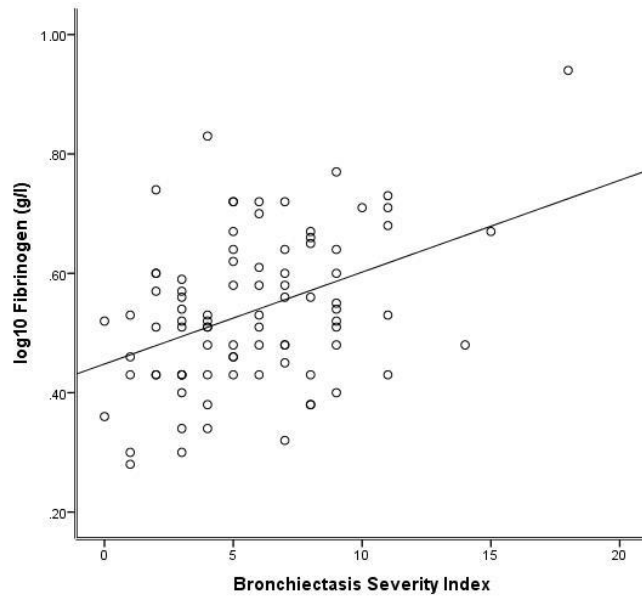


FIGURE 2: BSI Bronchiectasis Score (Figure 2A) and FACED Score (Figure 2B) correlate with serum fibrinogen concentration in the derivation cohort ($\rho = 0.34$, $p = 0.001$ and $\rho = 0.27$, $p = 0.014$ respectively).

2A:



2B:

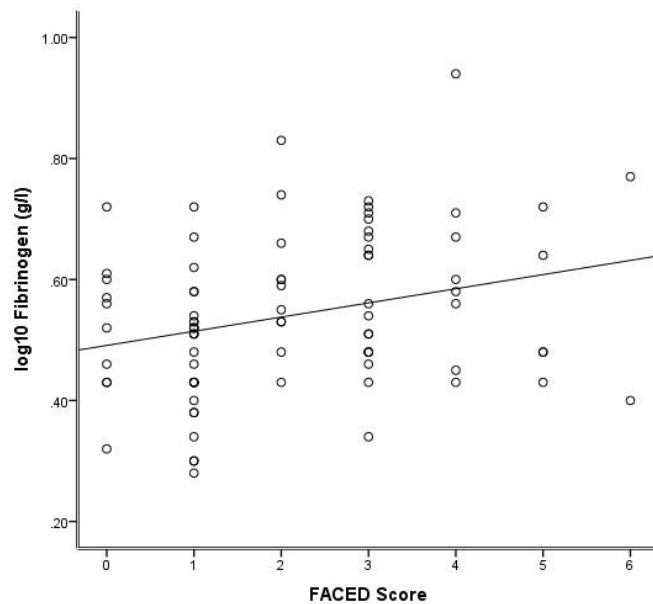


FIGURE 3: Radar plots of five serum inflammatory proteins by the three most common aetiologies of bronchiectasis in the whole derivation cohort (Figure 3A) and only those patients with moderate disease (Figure 3B). The blue line represents idiopathic patients, the purple line post-infectious patients and the black line patients with primary immunodeficiency. Numbers refer to quartiles of (\log_{10}) serum concentration.

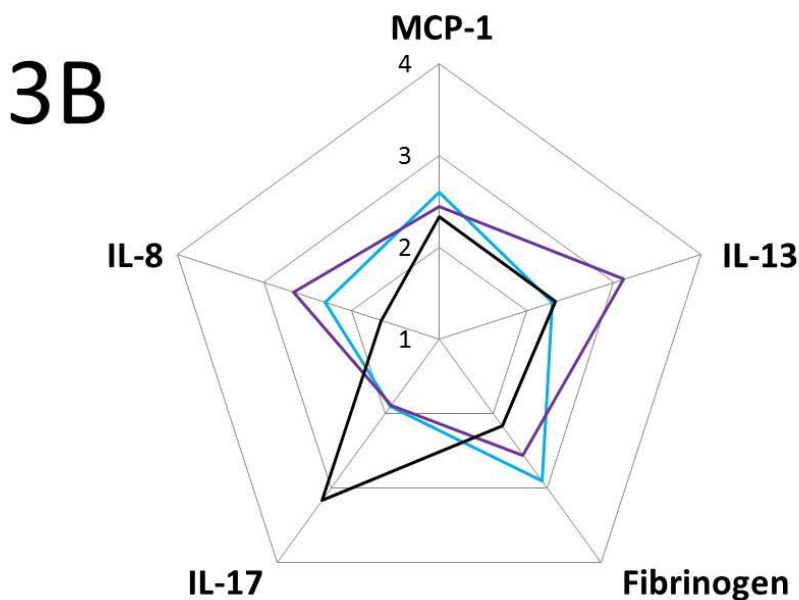
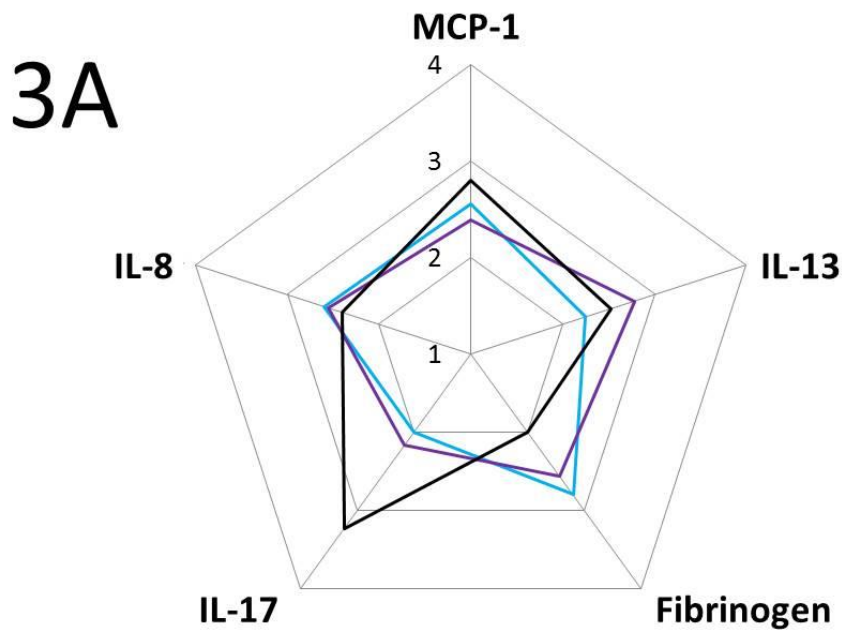


FIGURE 4: Receiver Operating Characteristic (ROC) curve for the ability of serum IL-17 concentration to differentiate primary immunodeficiency from other causes of bronchiectasis in the derivation cohort (AUC= 0.769 (95%CI) 0.661-0.877 $p<0.001$).

